بسم الله الرحمن الرحيم

SERRIOBIOCHEMICAL EFFECTS OF SODIUM NITRATE IN RATS

BY

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DEDICATION

To my father soul
To my mother,
To all my brother and sister,
To all my friend.

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All thanks and praises be to Allah, the lord of the mankind and all existing creatures. So the prayers and peace be up on the Mercy prophet; Mohammed.

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ABSTRACT

The present study was carried out to investigate the effect of sodium nitrate on Wister Albino rats.

Twenty rats were divided randomly into 4 groups, 5 rats each. Group A saved as control and group B, C and D were administrated orally with sodium nitrate ($NaNO_3$) at a dose of 50, 100 and 200 mg/kg body weight (Bwt) respectively.

There were no significant differences in body weights of treated groups compared to the control. However, the body weights of the control was increased at rate of 13.0%, while the rate of increase in body weights in groups (B) which was treated with 50 mg/Kg (Bwt) sodium nitrate was 3.4%. The increase in body weights of group (C) which was treated with 100 mg/Kg (Bwt) sodium nitrate was 1.8% and the increase in body weights of group (D) which was treated with 200 mg/Kg (Bwt) sodium nitrate was 4.15%.

There were a significant reduction in the number of RBCs. Of the treated groups compared to the control.

Hb concentration was significantly lower of treated groups compared to the control. There was significant decrease in PCV of treated groups compared to the control. Never the less there were significant increases in MCH value in treated groups compared to the control. There were significant decreases in total protein of treated groups compared to the control while the activity of ALP showed significant increase in the treated groups compared to the control.

There was no significant different among the group in MCV, MCHC, Albumin and Globulin concentration in treated groups compared to the control.

ملخص الأطروحة

في هذه الاطروحة تمت دراسة تحوي توضيح تأثير نترات الصوديوم علـى فئـران ويستر البيضاء.

قسمت عشرون فأراً عشوائياً إلي أربعة مجموعات في كل مجموعة خمـس فئـران المجموعة (أ) استخدمت كمجموعة ضبط والمجموعات (ب)، (ج) و(د) مجموعات معالجة تم تجريعهم بنترات الصوديوم بالفم على جرعات 50، 100 و200 ملجم/كجم على التوالي.

لم يكن هناك تأثير على وزن الجسم في المجموعات المعالجة بمقارنة مع مجموعـة الضبط بينما نجد أن وزن الجسم بالنسبة لمجموع الضبط زاد بنـسبة 13% بينمـا زاد وزن الجسم بالنسبة للمجموعه (أ) والتي تم تجريعها 50 ملجم/كجم نترات صوديوم بنسبة 3.4%. المجموعة (ج) والتي تم تجريعها 100 ملجم/كجم نترات صوديوم زاد وزن الجـسم عنـدها بمعدل 9.15% والمجموعة (د) والتي تم تجريعها 200 ملجم/كجم نترات صوديوم زاد وزن الجسم عندها بمعدل 1.8%.

حدث انخفاض معنوي في كريات الدم الحمراء في المجموعات المعالجة مقارنة مـع مجموعات الضبط من ناحية أخري حدث انخفاض معنوي في تركيز هيموقلوبين الـدم فـي المجموعات المعالجة مقارنة مع مجموعة الضبط. هناك انخفاض معنوي في حجـم الكريـة المضغوطة في المجموعات المعالجة مقارنة مع مجموعة الضبط ايضاً هناك زيادة معنوية في متوسط حجم هيموقلوبين الخلية في المجموعات المعالجة مقارنة مع مجموعة الضبط.

كما أن هناك انخفاض معنوي في البروتين الكلي لمـصل الـدم بـين المجموعـات المعالجة ومجموعة الضبط بينما نجد أن هناك زيادة معنويـة فـي أنـزيم ALP بالنـسبة للمجموعات المعالجة مقارنة مع مجموعة الضبط.

ليس هناك تغيير معنوي في متوسط حجم الخلية ومتوسط تركيز هيموقلوبين الخليـة في المجموعات المعالجة مقارنة مع مجموعة الضبط.

كما أنه ليس هناك تغيير معنوي في البيومين وقلوبيولين مصل الدم في المجموعـات المعالجة مقارنة مع مجموعة الضبط.

INTRDUCTION

Water has a profound influence on human health. At a very basic level, a minimum amount of water is required for consumption on a daily basis for survival; therefore access to some form of water is essential for life. However, water has much broader influences on health and wellbeing and this quantity and quality of the water supply are important in determining the health of individuals and whole communities (Esrey *et al*., 1995).

The first priority must be to provide access for the whole population to some form of improved water supply. However, access may be restricted by low coverage, poor continuity, insufficient quantity, poor quality and excessive cost relative to the ability and willingness to pay. Thus, in terms of drinking-water, all these issues must be addressed if public health is to improve. Water quality aspects, whilst important, are not the sole determinant of health impacts (Esrey *et al*., 1995).

The quality of water does, however, have a great influence on public health; in particular the microbiological quality of water is important in preventing ill-health. Poor microbiological quality is likely to lead to outbreaks of infectious water-related diseases and may cause serious epidemics to occur (Esrey *et al*., 1995).

Chemical water quality is generally of lower importance as the impact on health tends to be chronic long-term effects and time is available to take remedial action. Acute effects may be encountered where major pollution event has occurred or where levels of certain

chemicals are high from natural sources, such as fluoride, or anthropogenic sources, such as nitrate (Esrey *et al*., 1995).

The objective of this study was carried out to investigate the effect of sodium nitrate on wister albino rats.

CHAPTER ONE
LITERATURE REVIEW

1.1 Nitrites and Nitrates:

Nitrate and nitrite are naturally occurring ions that are part of the nitrogen cycle. The nitrate ion ($N0_3$) is the stable form of combined nitrogen for oxygenated systems. Although chemically uncreative, it can be reduced by microbial action. The nitrite ion (NO_2) contains nitrogen in a relatively unstable oxidative state. Chemical and biological processes can further reduce nitrite to various compounds or oxidize it to nitrate (ICAIR, 1987).

1.2 Environmental fate:

In soil, fertilizers containing inorganic nitrogen and wastes containing organic nitrogen are decomposed to give ammonia, which is then oxidized to nitrite and nitrate. The nitrate is taken up by plants during their growth and used in the synthesis of organic nitrogenous compounds. Surplus nitrate readily moves with the groundwater (EPA, 1987).

Under aerobic conditions, nitrate percolates in large quantities into the aquifer because of the small extent to which degradation or denitrification occurs. Under anaerobic conditions, nitrate may be denitrified or degraded almost completely to nitrogen. The presence of high or low water tables, the amount of rainwater, the presence of other organic materials and other physicochemical properties are important in determining the fate of nitrate in soil (Van and Loch, 1983). In surface water, nitrification and denitrification may occur, depending on the temperature and pH. The uptake of nitrate by plants,

however, is responsible for most of the nitrate reduction in surface water.

Nitrogen compounds are formed in the air by lightning or discharged into it from industrial processes, motor vehicles, and intensive agriculture. Nitrate is present in air primarily as nitric acid and inorganic aerosols, as well as nitrate radicals and organic gases or aerosols. These are removed by wet and dry deposition (WHO, 2003).

1.3 Uses

Nitrate is used mainly as inorganic fertilizers. It is also used as an oxidizing agent and in the production of explosives. Purified potassium nitrate is used for glass making while sodium nitrite is used as a food preservative, especially in cured meats. Nitrate is sometimes added to food to serve as a reservoir for nitrite (WHO, 2003**).**

1.4 Source:

1.4.1 Water:

Concentrations of nitrate in rainwater up to 5 mg/liter have been observed in industrial areas (Van and Matthijsen, (1989). about 90% of major epidemics in the Sudan are water- borne and were said to, cause the death of some 40% of children under five years of age (ELtyeb, 2002).

1.4.2 Food

Vegetables and cured meat are in general the main source of nitrate and nitrite in the diet, but small amounts may be present in fish and dairy products. Meat products may contain <2.7945 mg of nitrate per kg and <0.2-6.4 mg of nitrite per kg while dairy products may

contain <3-27 mg of nitrate per kg and <0.2-1.7 mg of nitrite per kg (ECETOC, 1988). Several vegetables and fruits may contain 200-2500 mg of nitrate per kg (Van and Matthijsen, 1989). The nitrate content of vegetables can be affected by processing, the use of fertilizers, growing conditions, especially the soil temperature and day light intensity (Gangolli, 1994). Vegetables such as beetroot, lettuce, radish, and spinach often contain nitrate concentrations above 2500 mg/kg, especially when they are cultivated in greenhouses. The nitrate level of vegetables that have been damaged, poorly stored, or stored for extended periods as well as pickled or fermented vegetables may be, up to 400 mg/kg have been found. Nitrite levels in food are very low, generally well below 10 mg/kg and rarely exceed 100 mg/kg. (WHO, 1995).

1.4.3 Air

The normal atmospheric nitrate concentrations ranging from 0.1 to 0.4μ g/m^3 have been reported (Prospero and Savoie, 1989), however, higher concentrations ranging from 1 to 40μ g/m3 have also been reported (Janssen *et al*., 1989). Indoor nitrate aerosol concentrations of 1.1-5.6μ g/m^3 were found to be related to outdoor concentrations (Yocom, 1982).

1.5 Kinetic and metabolisms of nitrate:

Ingested nitrate is readily and completely absorbed from the upper small intestine. Nitrite may be absorbed directly from both the stomach and the upper small intestine. Part of the ingested nitrite reacts with gastric contents prior to absorption (Walker, 1995).

Nitrate is rapidly distributed throughout the tissues. Approximately 25% of ingested nitrate is actively secreted into saliva,

where it is partly (20%) reduced to nitrite by the oral microflora. Nitrate and nitrite is then swallowed and re-enter the stomach. In human bacterial reduction of nitrate may take place in other parts of the gastrointestinal tract, but not in the stomach, however, potential reduction of nitrate in the stomach was reported in humans with low gastric acidity, in artificially fed infants, patients in whom hydrochloric acid secretion is slower, or patients using antacids (Colbers *et al*., 1995). In rats, active secretion and reduction of nitrate in saliva are virtually absent (Walker, 1995).

Absorbed nitrite is rapidly oxidized to nitrate in the blood nitrite in the bloodstream is involved in the oxidation of Hb to met Hb which can lead to cyanosis. The Fe^{2+} present in the haem group is oxidized to Fe^{3+} form, and the remaining nitrite binds firmly to this oxidized haem. The Fe^{3+} form does not allow oxygen transport, owing to the strong binding of oxygen (Jaffe, 1981; National Research Council, 1995).

Nitrite has been shown to cross the placenta and cause the formation of fetal methaemoglobinaemia in rats. It may react in the stomach with nitrosatable compounds (e.g. secondary and tertiary amines or amides in food) to form N-nitroso compounds. Such endogenous nitrosation has been shown to occur in human as well as animal gastric juice both *in vivo* and *in vitro,* mostly at higher pH values, when both nitrite and nitrosatable compounds were present simultaneously (Shephard, 1995; WHO, 1996).

The major part of the ingested nitrate is eventually excreted in urine as nitrate, ammonia, or urea, faecal excretion being negligible. (WHO, 1985; ICAIR, 1987; Speijers *et al*., 1989).

The excess nitrate excretion that has often been observed after low nitrate and nitrite intake originates from endogenous synthesis, which amounts, in normal healthy humans, to I mmol/day corresponding to 62 mg of nitrate per day or 14 mg of nitrate-nitrogen per day. Gastrointestinal infections greatly increase nitrate excretion, as a result, of increased endogenous (non-bacterial) nitrate synthesis, probably induced by activation of the mammalian reticuloendothelial system (WHO, 1996; Wishnok, 1995).

1.5.1 Endogenous Nitrate:

A major pathway for endogenous nitrate production is conversion of arginine by macrophages to nitric oxide and citrulline, followed by oxidation of the nitric oxide to nitrous anhydride and then reaction of nitrous anhydride with water to yield nitrite. Nitrite is rapidly oxidized to nitrate so reacted with Hb. In addition to macrophages, other cell types can form nitric oxide, generally from arginine. Under some conditions, bacteria can form nitric oxide by reduction of nitrite. These processes can lead to nitrosation of amines at neutral pH, presumably by reaction with nitrous anhydride. *In vitro* and *in vivo* studies showed that nitrate can be reduced to nitrite by bacterial and mammalian metabolic pathways, via the widespread nitrate reductase (Gangolli, 1994). In humans, saliva is the major site for the formation of nitrite. About Direct correlation between gastric pH, bacterial colonization, and gastric nitrite concentration has been observed at pH value from 1 to 7 in healthy people (Mueller, 1983).

In individuals with gastrointestinal disorders and achlorhydria, high levels (6 mg/liter) of nitrite in the blood can be reached (Rudell, 1978; Dolby, 1984). Infants younger than 3 months may be highly

susceptible to gastric bacterial nitrate reduction, as the pH is generally higher than in adults (Speijers *et al*., 1989). However, the presence of acid-producing lactobacilli in the stomach may be important, as these organisms do not reduce nitrate and may maintain a pH low enough to inhibit colonization by nitrate-reducing bacteria (Bartholomew, 1980).

1.6 Toxicity:

Toxicity of nitrates and nitrites (NO_3 and NO_2) are linked closely as causes of poisoning. NO_2^- is about 5-6 times more toxic than NO_3^- to ruminants. Ruminal microbes can reduce NO_3^- to NO_2, an intermediate step in the conversion of NO_3 to NH_3. Toxicity is less likely if rumen NO_3 is not reduced to NO_2, or if complete reduction to NH_3 is rapid. Sheep are less prone to poisoning than cattle. (Gregory *et al*., 1999).

The toxicity of nitrate to humans is mainly attributable to its reduction to nitrite. The major biological effect of nitrite in humans is its involvement in the oxidation of normal Hb to met Hb, which is unable to transport oxygen to the tissues. The reduced oxygen transport becomes clinically manifested when met Hb concentrations reach 10% of normal Hb concentrations .The above Methaemo-globinaemia, causes cyanosis and, at higher concentrations, asphyxia (WHO, 1996).

The Hb of young infants is more susceptible to met Hb formation than of older children and adults due to the large proportion of fetal Hb still present in the blood of these infants. In addition, there is a deficiency in the met Hb reductase responsible for the reduction of met Hb back to Hb. Under certain conditions, a higher reduction of nitrate to nitrite by gastric bacteria due to the low production of gastric

acid (WHO, 1996). Gastrointestinal infections increase the risk of higher yield of nitrite and thus a higher met Hb formation (Schuddeboom, 1995; WHO, 1996).

Pregnant women and people deficient in glucose-6-phosphate dehydrogenase or met Hb reductase are more Susceptible to met Hb formation (Speijers *et al*., 1989).

The reproductive behavior of guinea-pigs was impaired only at very high nitrate concentrations (30 000 mg of potassium nitrate per litre); the no-observed-effect concentration (NOEC) was 10000 mg/liter. In rabbits, dose levels of 250 or 500 mg of nitrate per liter administered during 22 weeks revealed no detrimental effects on reproductive performance after successive gestations. In sheep and cattle, no abortions were observed at dose levels causing severe methaemoglobinaemia (Speijers *et al*., 1989; WHO, 1996).

Nitrite appeared to cause fetotoxicity in rats at drinking-water concentrations equivalent to 200 and 300 mg of sodium nitrite per kg of body weight per day, causing increased maternal met Hb levels. However, similar doses in feed caused no embryo toxic effects in rats. In a reproductive toxicity study in guinea-pigs at dose levels of 0, 50, or 60 mg of sodium nitrite per kg of body weight per day given by subcutaneous injection, fetal death followed by abortion occurred at the highest dose level. Teratogenic effects were not observed in mice and rats (Speijers *et al*., 1989; WHO, 1996).

1.7 Mutagenicity:

Nitric oxide is mutagenic towards bacteria and human cells in culture. It causes DNA strand breaks, deamination probably via

nitrous anhydride, and oxidative damage. It can activate cellular defense mechanisms (Wishnok, 1995).

Nitrate is not mutagenic in bacteria and mammalian cells *in vitro.* Chromosomal aberrations were observed in the bone marrow of rats after oral nitrite uptake, but this could have been due to exogenous N-nitroso compound formation.

Nitrite is mutagenic. It causes morphological transformations in *in vitro* systems; mutagenic activity was also found. The results of *in vivo* experiments were controversial (Speijers *et al*., 1989; WHO, 1996).

1.8 Carcinogenicity:

Nitrate is not carcinogenic in laboratory animals. Some studies in which nitrite were given to mice or rats in the diet showed slightly increased tumor incidence. In other studies in which high levels of nitrite and simultaneously high levels of nitrosatable precursors when administered, increase tumor incidence was seen (Speijers *et al*., 1989; WHO, 1996). The types of tumors could be characteristic of the presumed corresponding N-nitroso compound endogenously formed. However, this increase in tumor incidence was seen only at extremely high nitrite levels, in the order of 1000 mg/litre of drinking-water. At lower nitrite levels, tumour incidence resembled those of controls in the treated groups with the nitrosatable compound only. Nitrite was shown to react with nitrosatable compounds in the human stomach to form N nitroso compounds. Many of these N-nitroso compounds have been found to be carcinogenic in all the animal species tested, although some of the most readily formed compounds, such as N-nitrosoproline, are not carcinogenic in humans. The N-nitroso

compounds carcinogenic in animal species are probably also carcinogenic in humans. There is link between cancer risk and endogenous nitrosation as a result of high intake of nitrate and/or nitrite and nitrosatable compounds (Speijers *et al*., 1989; WHO, 1996).

There is an association between high nitrate intake and gastric and/or esophageal cancer (NAS, 1981). However, exogenous nitrite intake, largely from preserved meat, was significantly associated with the risk of developing gastric cancer (ECETOC, 1988). On the other hand, studies based on food frequency questionnaires tend to show a protective effect of the estimated nitrate intake in vegetable and fruits on gastric cancer risk. Most likely these have a protective effect (Moller, 1995; WHO, 1996). Of nitrate from sources other than vegetables, such as the concentration in drinking-water or occupational exposure to nitrate dusts, have not shown a protective effect against gastric cancer risk. Other types of cancer have no association with nitrite or nitrate intake (Gangolli, 1994; Moller, 1995; WHO, 1996).

It has been established that the intake of certain dietary components present in vegetables, such as vitamins C and E, decreases the risk of gastric cancer. This is generally assumed to be at least partly due to the resulting decrease in the conversion of nitrate to nitrite and in the formation of N-nitroso compounds. 1t is possible that any effect of a high nitrate intake *per se* is masked in correlation studies by the antagonizing effects of simultaneously consumed dietary protective components (WHO, 1985).

1.9 Other effects:

It was believed that malformations have been related to high nitrate levels in drinking-water but studies failed to demonstrate a relationship between congenital malformations and nitrate intake (ECETOC, 1988). However Studies relating cardiovascular effects to nitrate levels in drinking-water gave inconsistent results (WHO, 1985).

Possible relationships between nitrate intake and effects on the thyroid have also been studied. Nitrate competitively inhibits iodine uptake. Epidemiological studies revealed indications for an antithyroid effect of nitrate in humans. If dietary iodine is available at an adequate range (corresponding to a daily iodine excretion of 150-300 µg/day), the effect of nitrate is weak, with a tendency to zero. The nitrate effect on thyroid function is strong if a nutritional iodine deficiency exists simultaneously (Horing *et al*., 1991).

Hettche (1956) described an association between high nitrate concentrations in drinking water and goiter incidence. As well Horing and Schiller (1987), Horing *et al*. (1991) and Van (I994) found that inorganic nitrate in drinking-water is factor of endemic goiter. There is a dose-response relationship of nitrate in drinking-water incidence of goiter as well as by (Van. (1994). both the experimental and epidemiological studies give the impression that nitrate in drinking-water has a stronger effect on thyroid function than nitrate in food. The differences in nitrate kinetics after ingestion through drinking-water and through food could be the cause of the difference in thyroid effects. However, there were no adequate studies to confirm this relationship. Furthermore, it was demonstrated that dietary iodine

deficiency is much more effective than nitrate exposure in causing goiter. Drinking water has a stronger effect on thyroid function than nitrate in food (Til, 1988; Kuper and Til, 1995).

CHAPTER TWO
MATERIAL AND METHODS

2.1 Material and experimental design:

2.1.1 Animals:

Twenty health y adult Wister Albino rats of both sexes, weight 78 - 80 g, were used. They were kept in cages within the Department of Biochemistry, Faculty of Veterinary Medicine, University of Khartoum. They were housed under standard environmental conditions with free access to food and water. They were kept for 10 days, as an adaptation period.

2.1.2 Experimental design:

At the end of the adaptation period, rats were divided randomly into 4 groups, 5 rats each. Group A saved as control; group B, C and D were administrated orally with sodium nitrate ($NaNO_3$) using nasogastric tube at a concentration rate of 50, 100, and 200 mg/kg body weight (Bwt) respectively for 21 days.

2.1.3 Parameters:

Clinical signs and mortality were recorded. Blood samples were obtained for hematological investigation which included red blood cells (RBC) counts, hemoglobin (Hb) concentration and packed cell volume (PCV). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated.

Serum investigation included the activities of alkaline phosphatase (ALP), total protein and albumin concentration globulin concentration was calculated.

Slices from liver, kidney and intestine were collected after death or slaughter and fixed in 10% neutral buffered formalin for histopathological investigation.

2.2 Methods:

2. 2.1 Hematological methods:

Blood samples were collected according to Schalm (1965) by puncturing retro- orbital plexus, with plain capillary tube in to dry clean tubes containing Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant. Blood samples were analyzed by Sysmex automated hematology analyzer KX-2 the sysmex KX-21 is an automatic multi parameter blood cell counter for invitro diagnostic use in clinical laboratories .The KX-21 employs three detector blocks to the kind of reagents for blood analysis. The WBC count was measured by the WBC detector block using the DC detection method. The RBC count and platelets were measured by the RBC detector block using the DC detector method. The HGM detector block measured the hemoglobin concentration using the non cyanide hemoglobin method. Blood was aspirated from the sample probe into sample rotor valve. 6 μl of blood measured by the sample rotor valve were transferred to the WBC transducer chamber along with 1.994 ml of diluents. At the same time 0.1 ml of WBC/Hb lyses was added to prepare 1:500 dilution samples.

When the solution was made in approximately 10 seconds, RBC was hemolyzed and platelets shrink, with WBC membrane held as they are. At the same time hemoglobin was converted into red colored methhemoglobin. Of the diluted/hemolyzed sample in the Hb

transducer chamber, approximately 1 ml was transferred to the Hb flow cell.

500 μL of sample in the WBC transducer were aspirated through the aperture. The pulses of blood cells when passed through the aperture were counted by the DC detecting method.

In the Hb flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LDE) was applied to the sample in the Hb flow cell. Concentration of the diluents alone that was measured before addition of the sample, thereby calculating hemoglobin value (Hb).

PCV evaluation by the analysis principle that RBC pulse height detection method (%) of whole RBC volume in whole blood

The Mean hemoglobin volume (pg) per RBC was calculated from Hb as follows:

2.2.2 Biochemical methods:

Blood samples were taken in a similar procedure as hematology in to dry clean tubes and allowed to clot at room temperature for 30 minutes then centrifuged (Hittch eba35) at 3000r.p.m.for10 minutes sera were separated and stored at (20 C) until analyzed for biochemical investigation.

2.2.2.1 Determination of serum total protein:

Serum total protein concentration was determined colorimetrically by Biuret method as described by Reinhold (1953).

Principle:

Copper in alkaline solution, reacts with the peptide bonds that link amino acids in protein producing a violet color. The intensity of color is proportional to the concentration of protein.

Changes in scale reading of colourmeter at wave length 540 nm were recorded.

Total protein Concentration =

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ X Concentration of standard}$$

Where the concentration of standard is 7mg/dl

2.2.2.2 Determination of serum albumin:

Serum albumin concentration was determined colorimetrically by Bromo Cresol Green (BCG) method described by Spencer and Price (1977).

Principle:

Bromo Crysol Green (BCG) solution, a yellowish indicator, reacts with serum albumin producing a blue green color. The intensity was proportional to the concentration of albumin present.

Changes in scale reading of colorimeter at wave length 600 nm were recorded.

Albumin Concentration =

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ X Concentration of standard}$$

Where the concentration of standard is 4g/dl

2.2.2.3 Determination of serum globulin:

This parameter is obtained by subtracting albumin from total protein concentration.

2.2.2.4 Determination of serum alkaline phosphatase:

Alkaline phosphatase activity was determined colorimetrically According to (Chemie, (1972).

Principle:

The p-nitro phenyl phosphate is hydrolyzed by alkaline phosphatase from the sample in the presence of magnesium ions, to form p-nitro phenol, which is yellow in color and can be read at 405nm. The intensity of color is proportional to concentration of ALP present in the sample.

The absorbencies were read four times for each sample, the change in absorbencies were then determined.

The Alkalin phosphatase activity was calculated by the following formula:

Alkaline phosphatase (Unit/L) = 2764x change of absorbance

2.3 Histopathology:

Slices from tissues were collected immediately after dissection of animal, fixed in 10% buffered formalin, embedded in paraffin wax, sectioned at 5µ and stained with heamatoxylin and eosin; according to the method of Duruy and Wallington. (1980).

2.4 Statistical analysis:

The data were subjected to completely randomized design. Analysis of variance (ANOVA) and mean separation were conducted to test significant difference of groups, according to Gomez and Gomrx (1984) with the aid of SAS computer program.

CHAPTER THREE
RESULTS

3.1 Clinical signs:

There were no clinical signs observed in rats of all groups throughout the experimental period.

3.2 Body weights:

The mean body weights of albino rats given different doses of sodium nitrate ($NaNO_3$) were presented in table (1).

There were no significant difference in body weights between the control and the treated groups. However, the body weights of the control was increased at rate of 13.0% while the rate of increase in body weights in groups (B) which was treated with 50 mg/Kg Bwt sodium nitrate was 3.4%, group (C) treated with 100 mg/Kg Bwt sodium nitrate the rate of increase was 1.8% and group (D) treated with 200 mg /Kg Bwt sodium nitrate at rate of 4.15%.

3.3 Hematological findings:

The hematological values in rats received sodium nitrate were summarized in table (2). There was significant reduction in the number of RBCs ($P > 0.05$) between the control and the treated groups. On the other hand the Hb concentration was significantly ($P > 0.05$) lower than the control.

There was a significant reduction ($P < 0.05$) of PCV values of the treated groups compared to the control; however, there were no significant differences MCV of the treated groups compared to the control.

Never the less the MCH was significantly (P> 0.05) increased in all treated groups compared to the control.

There were no significant differences in the MCHC values of the treated groups compared to the control.

3.4 Affect of sodium nitrate on some serum constituents:

The effects of sodium nitrate on the concentration of total protein, albumin, globulin and activity of ALP, were given in table (3).

There was a significant (P> 0.05) decrease in the level of total protein of the treated rats compared to the control. On the other hand there were on significant difference in Albumin and globulin concentration of the treated rats compared to the control. However in group (B) which was treated with 50 mg /Kg Bwt sodium nitrate albumin was reduced from 5.02 to 4.40 and also globulin decreased from 2.86 to 2.08 on day 21.

The albumin globulin ratio was decreased in the control from 4.39 to 2.46 but increased in the treated groups (B) from 1.83 to 2.14, group (C) from 1.54 to 2.24, group (D) from 2.23 to 2.32.

The activity of ALP showed significant (P> 0.05) higher in the treated group compared to control.

3.5 Histopathological findings:

The histopathological finding showed that the liver of rats in the control group and those received 50 and 100 mg\kgBwt sodium nitrate were normal, However, liver from rats that received 200mg\kgBwt sodium nitrate showed central hepatic fatty vaculation and congestion. The kidney from rats in control group and those received 50 and 200

mg\kgBwt sodium nitrate were normal, However kidney from rats received 100 mg\kgBwt sodium nitrate showed interstitial hemorrhage and tubular dilation. The intestine from rats in control and treated group were normal. (Figs. A, B, C

ملخص الأطروحة

في هذه الاطروحة تمت دراسة تحوي توضيح تأثير نترات الصوديوم علـــى فئـــران ويستر البيضاء.

قسمت عشرون فأراً عشوائياً إلي أربعة مجموعات في كل مجموعة خمـــس فئـــران المجموعة (أ) استخدمت كمجموعة ضبط والمجموعات (ب)، (ج) و(د) مجموعات معالجة تم تجريعهم بنترات الصوديوم بالفم على جرعات 50، 100 و200 ملجم/كجم على التوالي.

لم يكن هناك تأثير على وزن الجسم في المجموعات المعالجة بمقارنة مع مجموعـــة الضبط بينما نجد أن وزن الجسم بالنسبة لمجموع الضبط زاد بنـــسبة 13% بينمـــا زاد وزن الجسم بالنسبة للمجموعه (أ) والتي تم تجريعها 50 ملجم/كجم نترات صوديوم بنسبة 3.4%. المجموعة (ج) والتي تم تجريعها 100 ملجم/كجم نترات صوديوم زاد وزن الجـــسم عنـــدها بمعدل 9.15% والمجموعة (د) والتي تم تجريعها 200 ملجم/كجم نترات صوديوم زاد وزن الجسم عندها بمعدل 1.8%.

حدث انخفاض معنوي في كريات الدم الحمراء في المجموعات المعالجة مقارنة مـــع مجموعات الضبط من ناحية أخري حدث انخفاض معنوي في تركيز هيموقلوبين الـــدم فـــي المجموعات المعالجة مقارنة مع مجموعة الضبط. هناك انخفاض معنوي في حجـــم الكريـــة المضغوطة في المجموعات المعالجة مقارنة مع مجموعة الضبط ايضاً هناك زيادة معنوية في متوسط حجم هيموقلوبين الخلية في المجموعات المعالجة مقارنة مع مجموعة الضبط.

كما أن هناك انخفاض معنوي في البروتين الكلي لمـــصل الـــدم بـــين المجموعـــات المعالجة ومجموعة الضبط بينما نجد أن هناك زيادة معنويـــة فـــي أنـــزيم ALP بالنـــسبة للمجموعات المعالجة مقارنة مع مجموعة الضبط.

ليس هناك تغيير معنوي في متوسط حجم الخلية ومتوسط تركيز هيموقلوبين الخليـــة في المجموعات المعالجة مقارنة مع مجموعة الضبط.

كما أنه ليس هناك تغيير معنوي في البيومين وقلوبيولين مصل الدم في المجموعـــات المعالجة مقارنة مع مجموعة الضبط.

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Table (1) the mean body weights of albino rats given sodium nitrate

ose(mg/kg)	Zero day	21 day
0	80.18 ± 4.27	90.63 ± 5.55
50	100.6 ± 25.59	104.00 ± 7.74ns
100	78.82 ± 6.92	80.22 ± 5.62ns
200	78.97 ± 4.27	82.25 ± 10.14ns

Values are expressed as mean ± standard deviation.

Ns= no significance

Table (2): The hematological values in albino rats given sodium nitrate orally

Days	Dose (mg/kgBwt)	RBC10^6µm)	Hb (g/dl)	PCV	MCV (fl)	MCH (g/l)	MCHC (g/dl)
Zero	0	8.79±0.38	12.660±3.66	32.64±1.27	37.13±3.11	14.46±0.26	38.50±7.43
	50	9.34±0.15	13.820±2.87	46.68±4.51	48.91±3.34	14.86±0.22	29.61±98.74
	100	8.36±0.60	12.460±6.60	40.06±2.17	47.92±3.37	15.00±0.44	31.31±20.80
	200	9.20±0.63	13.360±6.96	39.82±2.88	43.28±4.53	14.52±0.28	40.41±24.36
21	0	8.92±0.27	12.90±0.40	33.06±2.20	37.06±0.68	14.46±0.32	39.01±23.22
	50	7.05±0.19^s	11.820±2.73^s	34.44±1.05^s	48.85±0.77^{ns}	16.76±0.33^s	34.32±4.68^{ns}
	100	5.91±0.45^s	10.360±7.19^s	28.74±2.09^s	48.63±0.71^{ns}	17.53±0.36^s	36.04±2.28^{ns}
	200	6.25±0.48^s	11.040±8.20^s	29.88±2.17^s	47.81±1.22^{ns}	17.60±0.35^s	36.94±11.33^{ns}

Values are expressed as mean ± SD (N= 5

Mean values having difference superscript letter (s) within each column are significantly different at ($P<0.05$).

ns = no significance.

Table (3): Effect of sodium nitrate on serum constituents in wister albino rats dosed with sodium nitrate orally

Days	Dose (mg/kg)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	ALP(u/l)
0	0	6.12±0.38	4.56±0.05	1.57±0.39	43.53±3.54
	50	7.88±0.18	5.02±0.17	2.86±0.26	22.46±2.56
	100	7.36±0.26	4.82±0.17	3.51±0.14	22.46±2.56
	200	7.16±0.21	4.95±0.15	2.22±0.21	25.98±3.97
21	0	7.07±0.04	5.02±0.06	2.05±0.04	47.91±5.67
	50	6.48±0.19^{s}	4.40±0.09^{s}	2.08±0.13s	46.44±8.71^{s}
	100	6.70±0.26^{s}	4.48±0.17^{ns}	2.22±0.36s	45.33±7.45^{s}
	200	6.44±0.18^{s}	4.42±0.21^{ns}	2.023±ns	37.04±5.07^{s}

Mean values having different superscript letter (s) with in each Colum is significantly different at ($p< .05$).

s =$p <.05$

ns =no significance

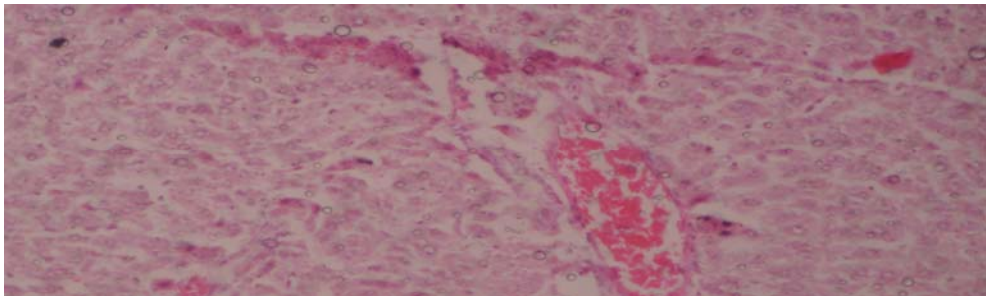

Fig. (A): Liver from rats received200mg/kgBwtsodium nitrate. Notice congestion and dilation of sinusoidal H α EX20

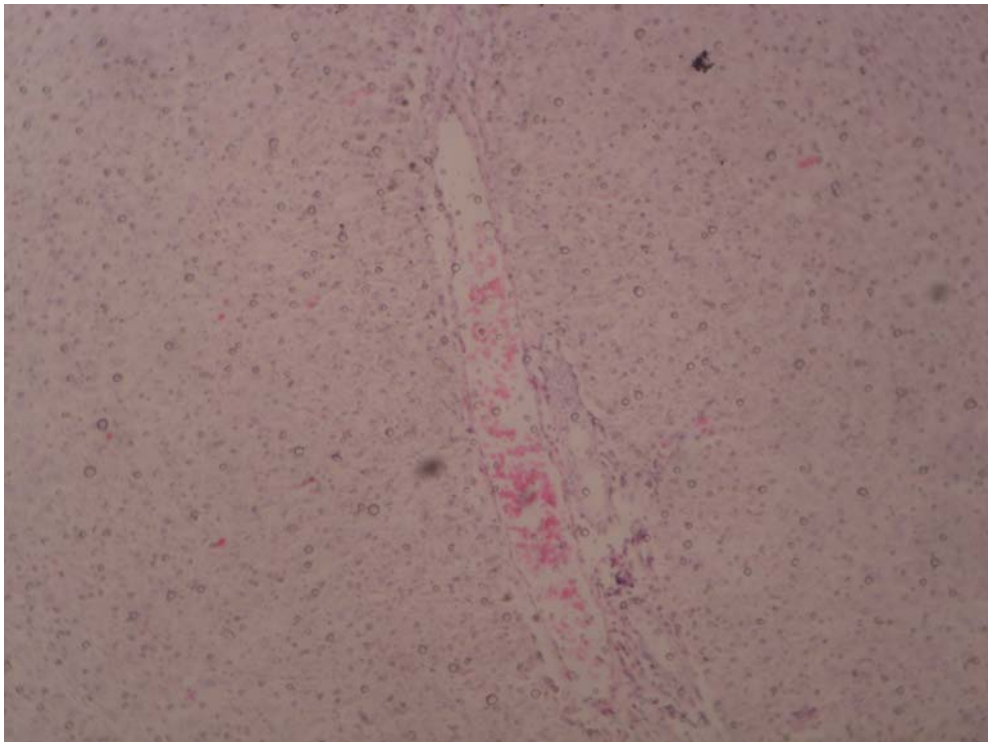

Fig. (B): Liver from rats received 200mg/kgBwt sodium nitrate. Notice mild congestion .H α EX20

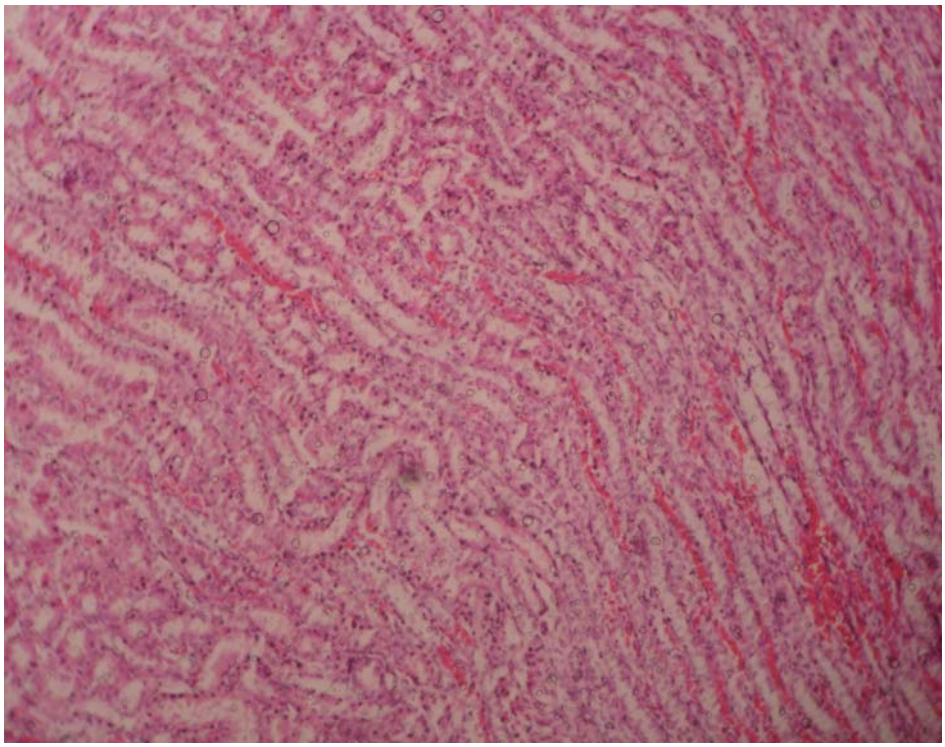

Fig. (C) Kidney from rats that received 100mg/kgBwt sodium nitrate. Notice sever intestinal hemorrhages and tubular dilation H α EX10

CHAPTER FOUR
DISCUSSION

In the present study there were no significant effects on the body weight in rats dosed with sodium nitrate ($NaNO_3$). This indicates that the sodium nitrate ($NaNo_3$) has no effect on the growth rate. This is in harmony with the Speijers *et al.* (1996) and WHO in (1996) who reported no change in body weight (Bwt) in animal dosed with sodium nitrate.

The decrease in Hb, PCV and RBC values in all treated rats in the third week because that sodium nitrate converted Hb to met Hb so Hb was lowered and PCV become small and RBCs decrease. In normal case red cells was protected from oxidative damage by the constant regeneration of reduced glutathione (GSH) via glutathione cycle. If the oxidative agent was increase, as in case of sodium nitrate, glutathione will be unable to protect red cell from this load of oxidative agent then sodium nitrate react with iron (fe^2) on red cell and converted it to (fe^{3+}) thus Hb converted to met Hb and hence Hb concentration was lowered. On the other hand the PCV and mature RBCs were decreased together with an increase immature RBCs. Also oxidative agent (sodium nitrate) oxidized the thiol and other group in globin chain that lead to the aggregation and precipitation of denatured Hb within the cell, so increase of MCV (Palliser, 1988).

Similar results were reported by Gregory and Ronald (1999) in who stated that Nitrate caused a significant reduction in RBCs and PCV in ruminants which ingested nitrate. The nitrate absorbed from digestive tract into the blood and converted the red pigment

hemoglobin to dark pigment met-Hb. Similar results were also recorded by Cheng and Chen (2002) who found that nitrate at a concentration of 105 mg NO_3 -N/l caused reduction of oxyhemocyanin and protein in individuals of the Kuruma shrimp Marsupenaeus japonicus Similarly, Cheng *et al*. (2002) studied nitrate accumulation (from $NaNO_3$) in tissues of the penaeid shrimp Penaeus monodon, and found that nitrate accumulated in muscle, hepato-pancreas, foregut, heart, gill, hemolymph, midgut and eyestalk over the ambient nitrate concentration.

American Academy of Pediatrics (2005) reported that Nitrates hazardous converted Hb to met-Hb and cause anemia in infant's age 3 month and younger. WHO (1999) reported that in human methaemoglobin levels were considerably higher than 2% of the total hemoglobin concentration. High levels of methaemoglobin were not restricted to infants only but were also prevalent in older age. Maximum levels were observed in all age, with the highest value in infants.

The decrease of total protein may be due to the change occurred in the hepatocytes which have been illustrated histopathologically since the hepatocytes are responsible for that production.

The elevated activity of ALP serum enzyme is may be due to congestion and dilation of sinusoi.

CONCLUSION AND RECOMMEMDATION

On the light of the present study it was concluded that nitrate in water significantly reduced the blood indices mainly RBCs, PCV and Hb. The liver damage by nitrate lead to reduction in total protein but the ratio between albumin and globulin were not affected.

According to the above mentioned results further studies are needed to:

1- Assess the long term exposure of NO_3

2- The relation between high NO_3 and hypothyroidism

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